SUPPORTING INFORMATION

Methylene Oxidation of Alkyl Sulfates by Cytochrome $P450_{BM-3}$ and a Role for Conformational Selection in Substrate Recognition

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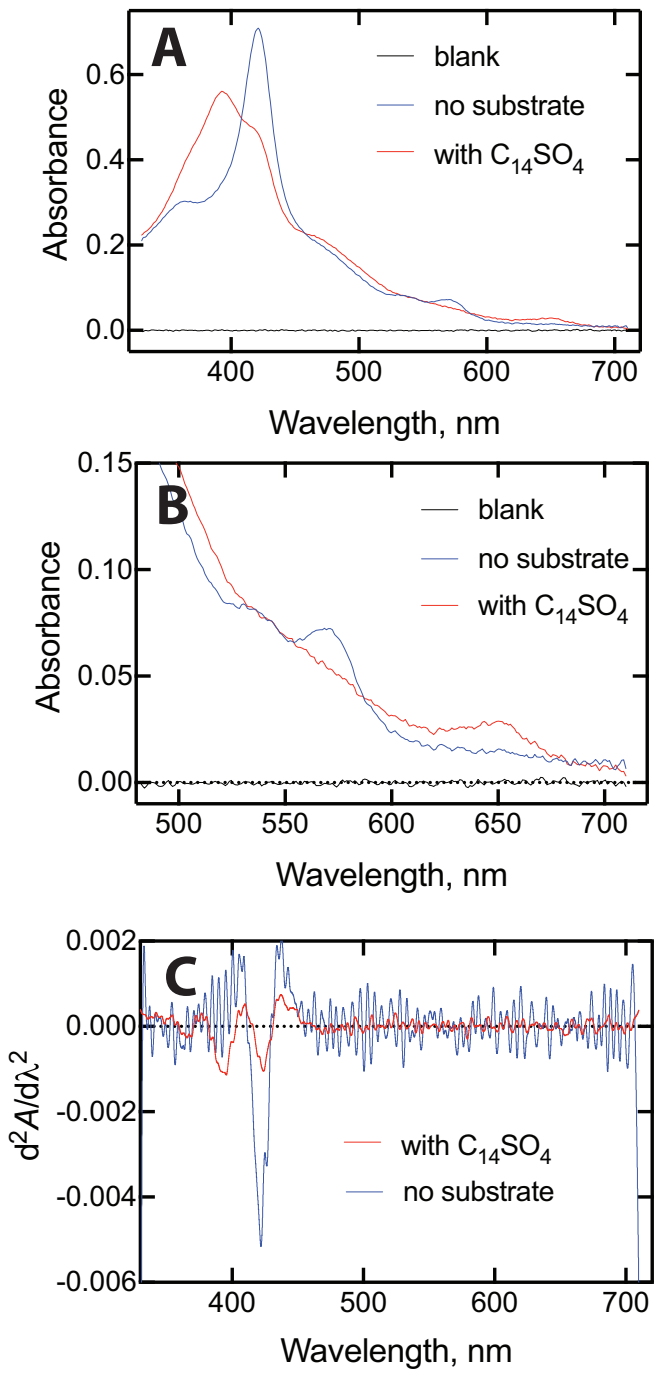
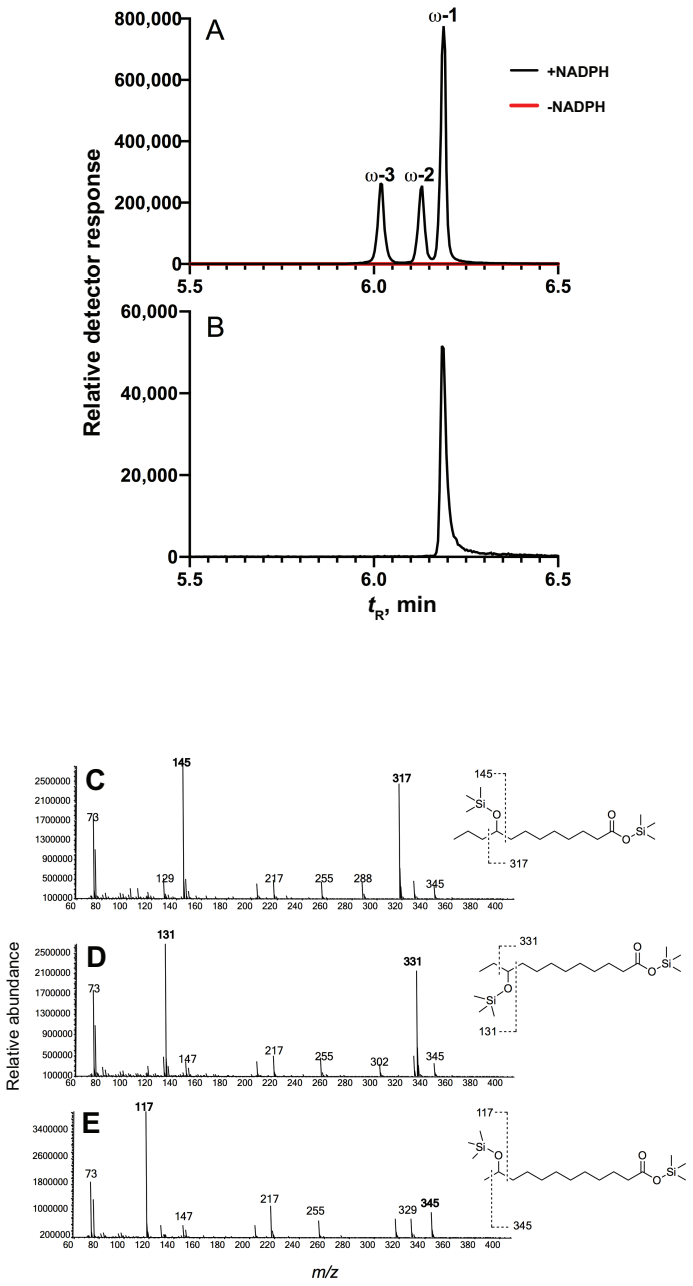
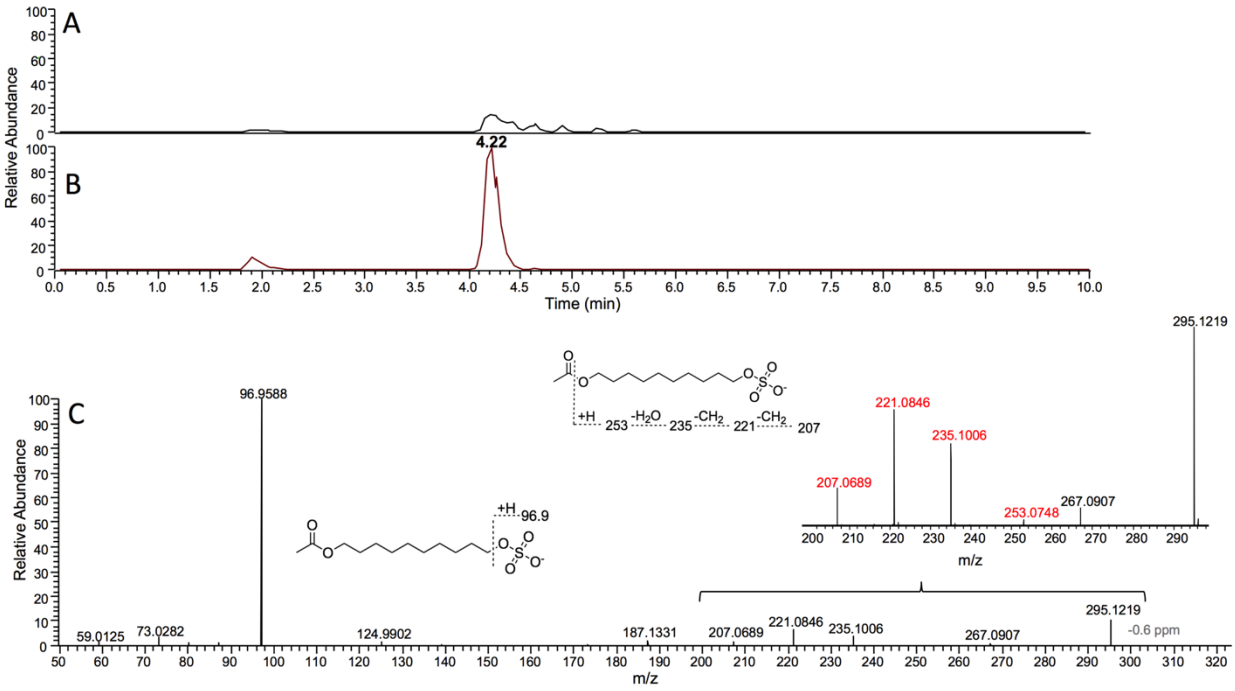


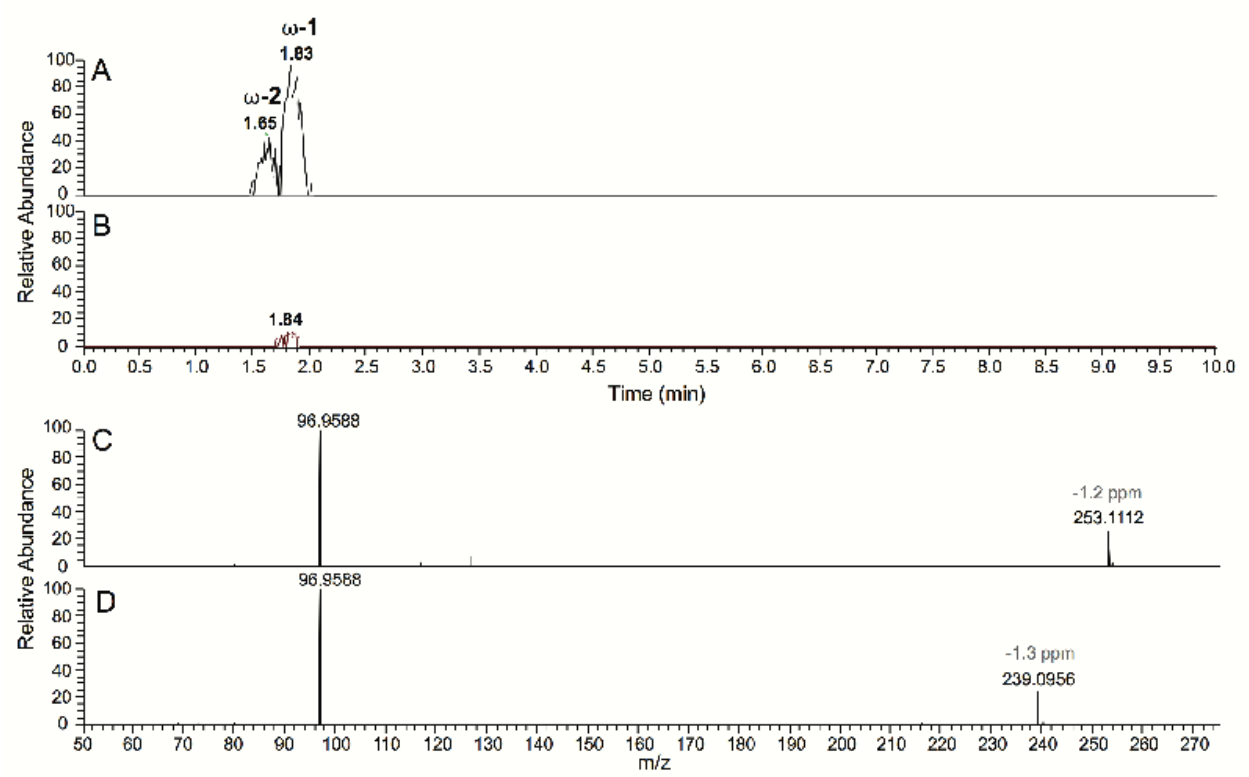
Supporting Figure S1. UV-visible spectra and second derivative spectra of $P450_{BM-3}$ without and with tetradecyl sulfate ($C_{14}SO_4$). All spectra were recorded with 5 µM $P450_{BM-3}$ in 50 mM potassium MOPS buffer (pH 7.4). **A.** Without (blue line) or with (red line) 50 µM tetradecyl sulfate ($C_{14}SO_4$). **B.** Part A expanded in the visible region. **C.** Second derivative spectra[1] of Part A (without substrate, blue line, filter 5 used for smoothing; with tetradecyl sulfate, red line, filter 19 used for smoothing).



Supporting Figure S2. Identification of lauric acid oxidation products formed by $P450_{BM-3}$ by GC-MS. **A.** Ion chromatogram resulting from incubation of lauric acid with $P450_{BM-3}$ and NADPH, following derivatization to trimethylsilyl products. The GC-MS traces (*m/z* 345, *vide infra*) are shown for the reaction in the presence (black trace) and absence (red trace) of NADPH. **B.** Ion chromatogram for authentic standard 11-hydroxy lauric acid, following derivatization to trimethylsilyl derivative. **C-E.** mass fragmentation spectra of reaction products (from Part A). **C**, *ω*-3 product; **D,** *ω*-2 product; **E**, *ω*-1 product.



Supporting Figure S3. LC-HRMS of $P450_{BM-3}$ reaction products of dodecyl sulfate following Baeyer-Villiger derivatization to esters. **A.** Dodecyl sulfate incubated with NADPH but without $P450_{BM-3}$ for 2 minutes. **B.** Dodecyl sulfate incubated with NADPH and $P450_{BM-3}$. Both reactions were followed by oxidation of alcohol products using Jones reagent[2] and then Baeyer-Villiger ketone oxidation[3] to form esters (Scheme 2). Extracted ion chromatograms for *m/z* 295.1221 are shown in Parts A and B, corresponding to product ester derivatives. **C.** HRMS (ESI^-) mass fragmentation spectrum of the ester formed from incubation of dodecyl sulfate with $P450_{BM-3}$ and NADPH followed by the chemical oxidation steps.



Supporting Figure S4. LC-HRMS of $P450_{BM-3}$ reaction products of dodecyl sulfate following Baeyer-Villiger derivatization to esters and base hydrolysis. **A.** Dodecyl sulfate incubated with NADPH and $P450_{BM-3}$ for 2 minutes. **B.** Dodecyl sulfate incubated with $P450_{BM-3}$ in the absence of NADPH. Both reactions were followed by oxidation of alcohol products using Jones reagent,[2] Baeyer-Villiger ketone oxidation[3] to form esters, and then NaOH treatment (Scheme 2). Extracted ion chromatograms for *m/z* 253.1115 and 239.0959 (combined) are shown in both Parts A and B, corresponding to the alcohol products detected after base hydrolysis. **C, D.** HRMS (ESI^-) mass fragmentation spectra of the alcohols formed from incubation of tetradecyl sulfate with $P450_{BM-3}$ and NADPH followed by the two chemical oxidation steps and base hydrolysis: **C**, fragmentation spectrum of the peak eluted at t_R 1.83 min peak; **D**, fragmentation spectrum of the peak eluted at t_R 1.65 min peak..

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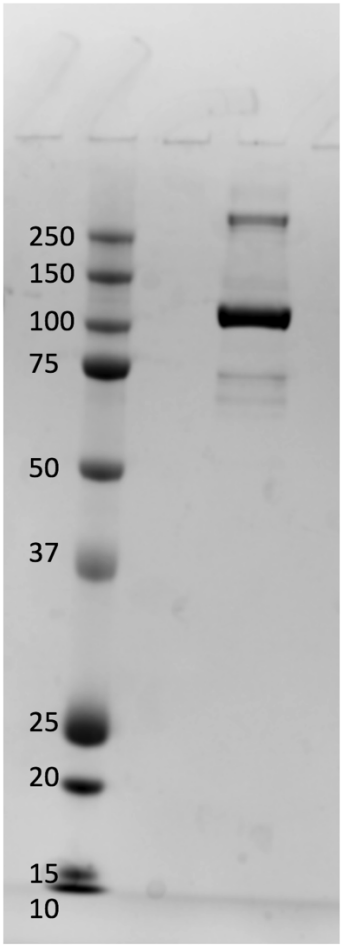
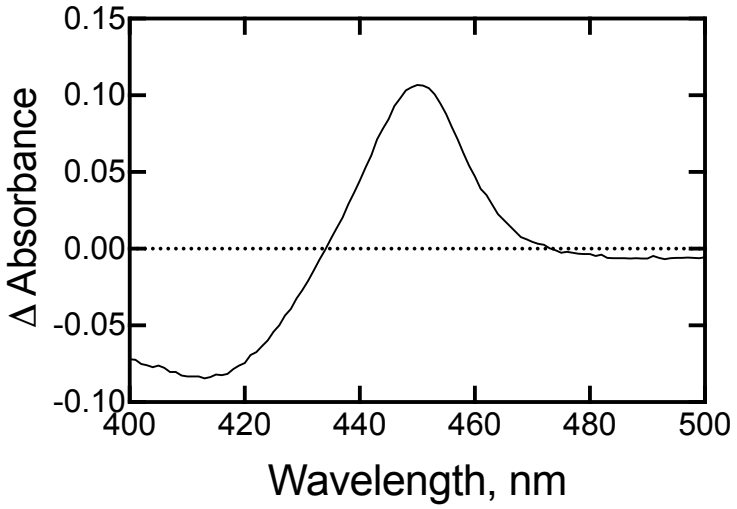


Supporting Figure S5. Purity of $P450_{BM-3}$ preparation determined using SDS-polyacrylamide gel electrophoresis (7.5%, w/v). The molecular weight standard markers (labeled as kDa) are on the left and the $P450_{BM-3}$ sample (10 pmol) is in the right lane. The higher band in the $P450_{BM-3}$ sample (~ 260 kDa) is presumed to be a dimer of the enzyme.



Supporting Figure S6. Fe^{2+}·CO complex *vs.* Fe^{2+} difference spectrum of $P450_{BM-3}$ preparation (1.25 μM) used for this study.

References

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